

1 **TO THE EUROPEAN PATENT OFFICE**

2 Re: Opposition to European Patent 0 125 023 (Genentech, Inc.)

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5 **DECLARATION OF JEFFREY V. RAVETCH, M.D., Ph. D.**

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8 I, Jeffrey V. Ravetch, M.D., Ph.D. declare and state as follows:

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10 1. I am a Professor at The Rockefeller University, Head of the Laboratory of Molecular
11 Genetics and Immunology, and an Adjunct Professor in the Department of Microbiology and
12 Immunology at Jefferson Medical College and Jefferson Cancer Institute. Prior to July 1996, I was an
13 Attending Physician in the Division of Hematologic Oncology (Department of Medicine) at Memorial
14 Hospital for Cancer and Allied Disease, and a Professor at Cornell University Medical College.

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16 2. In 1973, I acquired a Bachelor's Degree of Science in molecular biophysics/biochemistry
17 from Yale College. In 1978, I received a Ph.D. from the Laboratory of Genetics at The Rockefeller
18 University and in 1979, a Medical Doctorate from Cornell University Medical College. My education,
19 experience, patents, and publications are further set forth in the attached curriculum vitae (Exhibit "A").

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21 3. Since 1978, I have closely followed the academic literature regarding antibody structure
22 and synthesis. The authors of these works are predominantly molecular biologists with backgrounds
23 in immunology, immunologists and biochemists. Since 1979, my research and scholarship have
24 concentrated in molecular biology and immunology. Some of my scholarly endeavors pertain to
25 antibody structure. My laboratory has characterized immunoglobulin genes and Fc receptors. This

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1 research has included molecular cloning, chimeric antibody constructions, and other recombinant
2 antibody technology.

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4 4. I am familiar with the disclosure and claims of the above-identified European patent ("the
5 Cabilly patent"). I am also familiar with the opposition briefs filed by Boehringer Mannheim GmbH
6 (Opponent III), Protein Design Labs, Inc. (Opponent IV), Celltech Limited (Opponent VI), including
7 the submission dated February 20, 1997, filed by Opponent VI.

8
9 5. It is my understanding that certain Opponents question the sufficiency of the disclosure
10 provided in the Cabilly patent for making chimeric antibodies.

11 5.1 Opponent III specifically challenged the sufficiency of disclosure provided in the
12 examples since the Wagener *et al.* paper, cited on page 14, line 14 as describing the mouse hybridoma
13 cell line used as a source of the anti-CEA antibody mRNA, was not publicly available at the priority date
14 of the Cabilly patent.

15 5.2 Opponent IV pointed out that the restriction enzyme *Ava*III was not commercially
16 available at the priority date of the Cabilly patent and would have been difficult to purify. In addition,
17 according to Opponent IV the site-directed deletional mutagenesis step described in Example E.4 could
18 not have been performed at the priority date of the Cabilly patent since the Adelman *et al.* paper
19 referenced on page 21, lines 55-56 was published only at a later date.

20 5.2 In its submission dated February 20, 1997, Opponent VI suggests that the
21 preparation of chimeric antibodies is not sufficiently described in example E.4, since (1) the sequence
22 of the anti-CEA antibody was not available at the priority date of the above-identified patent; (2) the
23 vectors proposed for expressing the cDNA sequences encoding the chimeric light and heavy chains,
24 respectively contain the same origin of replication, which would lead to the ejection of one of these
25 vectors due to plasmid incompatibility, should one introduce them into a single bacterial host cell; and
26 (3) a "fully chimeric chain" could not have been prepared following the directions provided in E.4, since

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1 the mp7 vector described in the Messing *et al.* paper cited in the specification (Nucleic Acids Res. 9:309
2 (1981), cited on page 21, lines 54-55) does not contain an *Xba*I or *Pvu*II site in a cloning site.

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4 6. 6.1 I have reviewed the specification of the Cabilly patent, including examples E.1.1
5 through E.1.10 and E.4. Based upon my review and my general knowledge of the state of the art as it
6 existed in 1983, I disagree with the Opponents' position concerning the sufficiency of disclosure. It is
7 my considered scientific opinion that a chimeric antibody could have been prepared following the
8 directions provided in the Cabilly patent at the priority date of April 3, 1983.

9 6.2 Examples E.1.1 through E.1.10 describe the steps of the production of antibodies
10 to the carcinoembryonic antigen (CEA) ("anti-CEA antibodies") by recombinant DNA technology.
11 These steps include the isolation of messenger RNA (mRNA) for anti-CEA antibody light and heavy
12 chains (E.1.1), the cloning of cDNAs encoding anti-CEA antibody light and heavy chains (E.1.2 - E.1.6),
13 the construction of expression vectors for the direct expression of anti-CEA antibody light and heavy
14 chains, respectively (E.1.7 and E.1.8), the recombinant production of anti-CEA antibody chains by
15 expression in *E. coli* cells transformed with expression vectors encoding the respective chains (E.1.9),
16 and the reconstitution of the antibody chains (E.1.10).

17 6.3 Example E.4 describes the preparation of a chimeric anti-CEA antibody which
18 comprises a murine anti-CEA antibody variable region and a human antibody constant region.

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20 7. 7.1 The first part of example E.4 describes the construction of an expression plasmid
21 for a chimeric anti-CEA antibody heavy chain comprising the coding sequence for the variable part of
22 the murine anti-CEA antibody γ 1 (IgG-1) chain and human γ 2 (IgG-2) constant region. The nucleotide
23 sequence encoding the human γ 2 chain was described by Ellison *et al.*, Proc. Natl. Acad. Sci. USA
24 79:1984 (1982) (Exhibit "B"). The complete nucleotide sequence encoding the murine anti-CEA
25 antibody γ 1 chain is shown in Figure 4. The mRNA encoding an anti-CEA antibody γ 1 chain could
26 have been readily isolated by a skilled molecular biologist at the priority date of the Cabilly patent,

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1 following the instructions provided in example E.1. The carcinoembryonic antigen (CEA) was a well
2 known marker for human colon cancer at the time. Numerous papers, including Accolla *et al.*, Proc.
3 Natl. Acad. Sci. USA, 77:563 (1980) (Exhibit "C"), Kupchik *et al.*, Cancer Res., 41:3306 (1981)
4 (Exhibit "D"), and Hedin *et al.*, Mol. Immunol. 19:1641 (1982) (Exhibit "E"), described hybridoma cell
5 lines producing anti-CEA monoclonal antibodies of high affinity. Accordingly, a researcher had a
6 number of hybridoma anti-CEA antibody producing cell lines available at the priority date of the Cabilly
7 patent from which mRNA encoding such antibody could have been isolated following routine
8 techniques, as described in example E.1. The construction of an expression plasmid for a chimeric anti-
9 CEA antibody heavy chain (pChimI) is described on page 21 of the patent, and is illustrated in Figure
10 11. Although the generation of the desired portion of the human $\gamma 2$ gene involved the use of the
11 restriction enzyme *AvaIII*, which could have been purified by techniques known at the time, the same
12 result could have been readily achieved by relying on other restriction sites present in the $\gamma 2$ chain.
13 Alternatively, for convenience, new unique restriction sites could have been created by site directed
14 mutagenesis through the use of appropriate short synthetic oligonucleotide sequences. As the plasmid
15 pChimI contains murine variable region and human constant region coding sequences, it will in fact
16 express a chimeric antibody heavy chain when transformed into appropriate recombinant host cells, e.g.
17 *E. coli*. The fact that, in the chimeric antibody chain expressed, the change from murine to human
18 sequences does not take place at the exact variable to constant region junction does not take away from
19 its chimeric nature. This is clearly expressed at page 21, lines 41-42 of the patent, where it is stated that
20 "pChimI will, in fact, express a chimeric heavy chain when transformed into *E. coli*, but one wherein
21 the change from mouse to human does not take place at the variable to constant region junction."
22 Indeed, there is no reason to believe that an antibody comprising such chimeric chain would not be fully
23 functional.

24 7.2 Figure 12 shows the further modification of the plasmid pChimI to provide a
25 plasmid (pChimII) containing the coding sequence of a second chimeric antibody heavy chain, in which
26 the change from murine to human sequences takes place at the exact variable to constant region junction.

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1 The construction of this second chimeric antibody heavy chain proceeds through an intermediate
2 plasmid (referred to as pIntermediate human/mouse hybrid in Figure 12), which contains a short
3 extraneous sequence, consisting of a small portion of the variable region of the human $\gamma 2$ heavy chain,
4 and a small portion of the constant region of the murine anti-CEA antibody $\gamma 1$ heavy chain. According
5 to the instructions provided at pages 21-23 and in Figure 12, this extraneous sequence is removed by
6 excising the *Xba*I to *Pvu*II fragment of the plasmid, cloning in an M13 phage vector, *in vitro* site directed
7 deletion mutagenesis to provide the correct junction sequence, followed by cloning of the modified,
8 correct *Xba*I to *Pvu*II fragment into the XAP plasmid to form pChimII. The complete nucleotide and
9 amino acid sequences of the murine anti-CEA $\gamma 1$ chain are shown in Figures 4 and 5. The nucleotide
10 and deduced amino acid sequences of human antibody $\gamma 2$ heavy chain constant region were described
11 in Ellison *et al.*, *supra* (Exhibit "B"). Based upon this information, a person of ordinary skill in the art
12 at April 8, 1983 would have well known that in the coding sequence present in the intermediate plasmid
13 shown in Figure 12 any segment present in addition to the coding sequence of the human antibody heavy
14 chain constant region and the murine anti-CEA antibody heavy chain variable region is extraneous, and
15 needs to be excised to obtain an accurate variable region - constant region junction in the chimeric
16 antibody heavy chain. A skilled person would also have known how to perform the excision. Site-
17 directed deletion mutagenesis was a technique known in the art at the priority date of the Cabilly
18 application. For example, Messing *et al.*, Nucleic Acids Res. 9: 309 (1981) (Exhibit "F"), cited at page
19 21, lines 54-55 of the Cabilly patent, teaches the use of M13 for site-directed mutagenesis in general.
20 A specific version of this technique, deletion mutagenesis, was also well known in the art at the priority
21 date - see, for example, Wallace *et al.*, Science 209:1396-1400 (1980) (Exhibit "G"). I note that the
22 Cabilly patent does not call for the use of the phage vector M13mp7 described by Messing *et al.*, or of
23 any other specific phage vector. It is clear to me that the instructions to clone the *Xba*I to *Pvu*II fragment
24 into "M13 phage as described by Messing *et al.*" (page 21, line 54, emphasis added) mean just what they
25 say, the cloning of the fragment into an M13-based expression vector, following the approach described
26 by Messing *et al.* In 1983, this could have been achieved in at least three ways, or by the combination

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1 of any of these three approaches. First, an M13 vector having a cloning site including the *Xba*I and
2 *Pvu*II restriction sites could have been constructed following the teaching of Messing *et al.* Second, the
3 ends of the *Xba*I-*Pvu*II fragment could have been modified by the ligation of synthetic adapters, which
4 would have enabled the insertion of this fragment into the cloning site of an existing M13 vector, such
5 as M13mp7. Third, restriction sites present in an existing vector, such as M13mp7, could have been
6 converted into the required *Xba*I and *Pvu*II restriction sites by site-directed *in vitro* mutagenesis. As
7 mentioned before, the latter technique was well known in the art at the priority date of the Cabilly patent,
8 and is also illustrated in Figure 3 of Messing *et al.* It is, therefore, my considered opinion that a person
9 of ordinary skill in the art at the priority date of the Cabilly application had the ability to prepare a
10 correct variable domain - constant domain junction in the coding sequence of the disclosed chimeric
11 immunoglobulin, without undue experimentation.

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13 8. According to example E.4 of the Cabilly application, the expression plasmid for a
14 chimeric antibody light chain is prepared by analogy to the preparation of the chimeric heavy chain
15 expression plasmid, and the two plasmids (harboring coding sequences for the chimeric heavy and light
16 chains, respectively) "are then double transformed into *E. coli* W3110, the cells grown and the chains
17 reconstituted as set forth in paragraph E.1-E.3 supra." (Page 51, lines 10-12.) In view of the disclosure
18 provided in examples E.1-E.3 and in the rest of example E.4, a person skilled in the art would have had
19 no difficulty at the priority date of the Cabilly application to perform these steps. Figures 2 and 3 of the
20 Cabilly application show the nucleotide and deduced amino acid sequences of mouse anti-CEA antibody
21 κ (light) chain. The nucleotide and amino acid sequences of human κ constant regions were well known
22 in the art at the priority date of the Cabilly application. A person of ordinary skill in the art could have
23 constructed a chimeric light chain expression vector on the analogy of construction of the chimeric
24 heavy chain expression vector. A skilled person could also have performed the subsequent steps of
25 transformation, cultivation, isolation, and reconstitution on the analogy of the detailed description

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1 provided for the preparation of non-chimeric recombinant antibodies. None of this steps would have
2 required any significant experimentation at the priority date of the Cabilly application.

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4 9. The experimental section describing the preparation of non-chimeric anti-CEA antibody
5 includes coexpression of the antibody heavy and light chains in the same *E. coli* host cell (page 19). The
6 data set forth in the table at page 19 and the Western blot shown in Figure 8C confirm that double
7 transformants have indeed been produced. According to page 22, lines 3-4, the two plasmids containing
8 the coding sequences of chimeric anti-CEA antibody heavy and light chains are "double transformed
9 into E. coli W3110, the cells grown and the chains reconstituted as set forth in paragraph E.1-E.3 supra."
10 A person working in this field could have performed the cotransfection and reconstituted the chimeric
11 chains, following the procedure which has been shown to be successful for non-chimeric antibodies.
12 Opponent VI cited no scientific support for the proposition that the introduction of the chimeric heavy
13 and light chain plasmids into a single bacterial host cell would have resulted in the ejection of one of
14 the chains due to plasmid incompatibility. Plasmid incompatibility plays a role primarily in the absence
15 of selection pressure. From the Cabilly patent it is clear that the double transformants (cotransformed
16 with immunoglobulin heavy and light chains, respectively) when maintained under selection pressure,
17 using different (ampicillin and tetracycline) selection markers resulted in the data shown in the Table
18 on page ^{19.}
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20 10. Following the procedure described in the examples, the recombinant anti-CEA
21 antibodies, including the chimeric antibodies, are obtained in the form of insoluble refractile bodies, and
22 need to be recovered and assembled by cell lysis, solubilization, and subsequent reconstitution. All
23 these steps are described in the Cabilly patent in a detailed manner. Accordingly, a person having the
24 usual skills in molecular biology laboratory techniques in 1983, could have followed the instructions
25 provided in the patent and obtain a chimeric antibody with antigen-specificity by reconstitution of the
26 chimeric antibody chains expressed in the same host cell or in separate host cells. However, based upon

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1 the totality of the disclosure I conclude that the inventors had also recognized the possibility of secreting
2 the produced antibodies into the periplasmic space of *E. coli*, or into the culture medium, if the host is
3 yeast or a gram positive bacterium (page 11, lines 3-17). Should I have read this passage in 1983, it
4 would have been clear to me that the way to achieve secretion is to replace the native signal sequence
5 of immunoglobulin chains by an *E. coli* signal sequence. A variety of *E. coli* signal sequences were
6 known by early 1983, as attested by the attached review paper: Watson, Nucleic Acids Res. 12:5145
7 (1984) (Exhibit "H"). Although the latter paper was published after the priority date of the Cabilly
8 patent, most references cited as describing various *E. coli* signals were published prior to or in 1983.
9 For example, the amino acid sequence of the signal peptide of ompA protein was published in 1980
10 (Movva *et al.*, J. Biol. Chem. 256:27 (1980) (Exhibit "I"). While the selection of the most efficient
11 signal sequence would have involved some experimentation, secretion of at least some chimeric anti-
12 CEA antibody, or antibody fragment (e.g. Fab protein) would have been expected. In addition, at page
13 8, lines 25-26, the Cabilly patent lists a number of further prokaryotic hosts suitable for antibody
14 production. Of these, the gram positive genus *Bacillus* was shown to secrete large amounts of certain
15 proteins into the medium shortly after the priority date of the Cabilly patent (Palva *et al.*, Gene 22:229
16 (1983) - Exhibit "J"). *Pseudomonas aeruginosa*, a gram negative bacterium, was also shown to secrete
17 various proteins into the medium (Liu, J. Infect. Dis. 130(Suppl):594 (1974)- Exhibit "K"). These
18 prokaryotes, which are specifically disclosed in the Cabilly patent, would have been further candidates
19 for secretion expression of antibodies or antibody chains or chain combinations.

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21 11. I have reviewed the Declaration of Marc J. Shulman, dated May 21, 1994 along with
22 Exhibits A to E (Shulman I Declaration). I have also reviewed a second Declaration signed by Dr.
23 Shulman on May 15, 1996, along with its Exhibits, including a draft grant application by Gabrielle
24 Boulianne bearing the handwritten date "FEB. '83" and the research report submitted by Dr. Shulman
25 to the Arthritis Society with a letter dated September 30, 1983.

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I did not attend the Ninth Annual Meeting of the Clinical Ligand Assay Society held between March 13 and 17, 1983 in Philadelphia, Pennsylvania, nor have I had the opportunity to speak to anyone who did. I have reviewed the copies of five slides that were reported to have been shown by Dr. Shulman at a lecture delivered at that meeting. The slide entitled "Construction and expression of an Ig Heavy Chain Gene" is a summary of work done by numerous workers in the field of molecular immunology from 1979-1982, including my own work on the structure of the human IgM gene. Indeed, I noticed that Dr. Boulianne refers to my group as the source of human C_κ and C_μ regions shown in the first slide (References 21-23 cited at page 4 of the Boulianne draft). The organization and rearrangement of the genetic elements is summarized in this slide and it contains no novel information, or any detail, statement or suggestion that would lead one to a method of constructing a chimeric antibody. Similarly, the slide entitled "μ Heavy Chain Gene" is a graphic illustration of the genetic organization of the rearranged gene that is found in B cells expressing the IgM antibody. Information summarized in this slide was well known by 1983 and provides no suggestion to prepare chimeric antibodies. The third slide in this series entitled "Expression System" is a schematic of the method published in late 1982 and early 1983 by two independent groups investigating the mechanisms of immunoglobulin gene expression. (Rice and Baltimore, Proc. Natl. Acad. Sci. USA 79:7862 (1982) - M9; Oi *et al.*, Proc. Natl. Acad. Sci. USA 80:825 (1983) - M10). The introduction of heavy or light chain genes into lymphoid cells, derived from myeloma, hybridoma or virally transformed B cell lines results in the expression of these transfected DNA molecules and their assembly with the endogenous heavy or light chain protein into an assembled immunoglobulin. No new information is provided in this figure, and there is nothing present that would be related to the construction and expression of chimeric antibodies. The final two slides in this series relate to "Engineering the V region (by) *In Vitro* Mutagenesis" and "Engineering the C region." These slides reiterate the methods of site-directed mutagenesis and restriction enzyme based methods for reconstructing genes, which were well known in 1983. While the final slide suggests the substitution of other C regions, there is no explanation of what kind of "other regions" would be substituted. Nothing in this slide indicates that the "other C region" could be a human constant region

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1 substituted for the constant region of a mouse antibody. For example, "engineering the C region" might
2 have meant the generation of immunoglobulin molecules in which the variable regions are identical but
3 the native constant regions are replaced by constant regions of other isotypes, as it was later done, and
4 published by Dangi *et al.* (The EMBO Journal 7:1989 (1988) - Exhibit "L"). There is no novel method
5 being suggested by these slides. No suggestion as to the vector that is to be used is provided, nor is there
6 sufficient detail to determine what Dr. Shulman was proposing to construct and how he was planning
7 to achieve his (unidentified) goal. Accordingly, based upon the slides a person skilled in the art of
8 antibody production in 1983 would not have had a clear understanding of what Dr. Shulman was
9 proposing, even less of how the proposed construct was to be made.

10 The draft research proposal does not appear to be a public document. It is nonetheless useful in
11 getting a better idea of the approach the laboratory of Dr. Shulman might have been contemplating to
12 construct chimeric immunoglobulin genes, although it is unclear whether any of this has been
13 communicated to the public before the priority date of the Cabilly patent. In the proposal, restriction
14 enzymes were proposed to cut the rearranged heavy and light chain genes in the intron separating the
15 assembled V region and the constant region and then recombined with constant regions derived from
16 cloned human genes. The reassembled genes are inserted into the pSV2 neo vector, which will permit
17 the stable transformation of a hybridoma cell line. Expression of the introduced chimeric gene will
18 depend on the presence of a promoter element on the transferred DNA. The publication which describes
19 the outcome of those experiments, in 1984 (Boulianne *et al.*, Nature 312:643 (1984) - M42), reveals that
20 Dr. Shulman restricted his approach to the expression of chimeric antibodies in lymphoid cells, since
21 those cells retained the transcriptional, translational and assembly machinery to permit the expression
22 of immunoglobulin genes. Similar studies, published in 1984 by Morrison *et al.* (Proc. Natl. Acad. Sci.
23 USA 81:6851 (1984) - M41) use a very similar approach and indicate some of the difficulties inherent
24 with this method.

25 Expression of immunoglobulin genes in lymphoid cells was found, in 1983, to depend not only
26 on the presence of a promoter upstream of the rearranged V region, but required the presence of an

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1 "intronic enhancer" residing in the intervening sequence between the assembled V region and the
2 constant region. The presence of this intronic enhancer is required for expression of the transfected
3 immunoglobulin gene. There is no indication in the slides allegedly shown by Dr. Shulman or the draft
4 grant application that inclusion of this element was considered in the construction of their chimeric
5 genes. Furthermore, as was explicitly discussed in the Morrison study, the use of cross-species
6 enhancers resulted in very inefficient expression of the transfected gene, suggesting that for the method
7 to work, the enhancer would have to be of the same species as the recipient lymphoid cell line.
8 Accordingly, even if the structure of an antibody having a human constant region fused to a murine
9 variable region had been clearly communicated in the slides, based upon the information shown, a
10 skilled person would not have been able to make the constructs with a reasonable expectation of success.
11 This is particularly so since the presence of an intronic enhancer is critical to the success of this approach
12 and no mention is made of this element. Therefore, I conclude that the slides of Dr. Shulman, even if
13 accompanied by a limited explanation of the structures shown and the methods contemplated, did not
14 provide sufficient information for the construction of a chimeric gene which would be able to express
15 in a lymphoid cell.

16 Finally, expression in *E. coli*, yeast or other non-lymphoid eukaryotic cells (which do not
17 ordinarily express an immunoglobulin) was specifically excluded by the approach proposed by Shulman,
18 since the immunoglobulin promoter/enhancer is specific for lymphoid cells and would not function
19 elsewhere. In this regard, I note that the phrase host cell, "which does not ordinarily express an
20 immunoglobulin" (present in Patentee's second subsidiary claim request) clearly means to me non-
21 lymphoid cells which consequently do not have the native machinery to produce immunoglobulins. A
22 lymphoid cell line which is deficient in antibody production would nonetheless be viewed as one which
23 ordinarily expresses immunoglobulin. The interpretation which has been placed on this phrase by
24 Celltech (letter February 20, 1997, section 3-4-3) is not one which would have been adopted by one
25 working in this field. It is clear to me from reading the Cabilly patent (for example from the discussion
26 on page 4, lines 2-25 of the disadvantages of using hybridoma or B cell lines) that part of the point of

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1 the Cabilly invention was to provide ways for using cells which do not ordinarily produce
2 immunoglobulins and are not "lymphoid" or "myeloid" or "immunocompetent."

3 In conclusion, I am of the opinion based on the lecture slides, grant application and subsequent
4 publications of Dr. Shulman (without suggesting in any way that any of this information was publicly
5 available at the priority date of the Cabilly patent) that the limited approach he seems to have
6 contemplated was an extension of the studies published in 1982/1983 utilizing transfection of
7 immunoglobulin genes which had been modified by the substitution of the constant region from a human
8 immunoglobulin gene. This approach was limited to a chimeric gene produced in lymphoid cells, and
9 the actual work, published in the December 1984 Boulianne *et al.* paper, was performed in Sp2/0
10 lymphoid cells, which have the native machinery to produce antibodies, although are actually deficient
11 in the production of both antibody heavy and light chains. None of the documents studied have any
12 suggestion that the production of chimeric antibodies in cells other than lymphoid cells was
13 contemplated or would have been expected to be possible given the specific specialization of lymphoid
14 cells for the expression and assembly of antibody molecules.

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16 12. I have read the grant application submitted by Dr. Leonard Herzenberg on 2/28/82
17 (Application No: 2 RO1 CA04681-24) entitled "Genetic Studies with Mammalian Cells." In this
18 application, Dr. Herzenberg summarizes the past progress of his research group on the analysis of
19 lymphoid cells and lymphoid cell proteins using the technique he pioneered, "fluorescence activated cell
20 sorting" or FACS. Dr. Herzenberg proposes experiments for the coming granting period that would
21 extend on his ongoing studies. In particular in his "Research Plan" he mentioned under specific aim 1
22 a goal of selecting interchromosomal (cross allotype) and interspecies (mouse-human) switch variants
23 producing chimeric antibodies. On pages 34-37 of this application the rationale and approach Dr.
24 Herzenberg is considering is described in somewhat greater detail, although still remains rather sketchy.
25 The experimental question which is being considered in this goal is the molecular explanation of the
26 appearance of immunoglobulin switch variants and, in particular, switches which appear to defy the

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1 then-known genetic structure of the heavy chain locus. Switching refers to the process wherein one
2 constant region is naturally substituted with another constant region while retaining the same assembled
3 variable region. This phenomenon occurs during the maturation of an immune response, where
4 antibodies of the IgM class are produced first, followed by IgG antibodies, with the same specificity for
5 antigen. This switching (isotype or class switching) was believed to proceed in an orderly fashion,
6 following the order of constant region genes on the chromosome, i.e. μ , δ , $\gamma 3$, $\gamma 1$, $\gamma 2\beta$, $\gamma 2\alpha$, ϵ , α .
7 Switching from μ to $\gamma 1$ results in the deletion of the $\gamma 3$ gene. Dr. Herzenberg identified a switch variant
8 that appeared to go backwards, that is from expressing $\gamma 2\beta$ to $\gamma 1$. He wished to understand how such
9 a switch was possible and proposed testing the hypothesis that this switch involved interchromosomal
10 rearrangement, in contrast to the usual intrachromosomal rearrangement. As he states "our major
11 impetus, however, derived from the potential we saw in such variants for answering a variety of
12 questions about antibody molecules and the genetic mechanisms that control their production."

13 To address this question, Dr. Herzenberg proposed to select for naturally occurring switch
14 variants using hybridoma fusions between lymphoid cells which would allow him to distinguish between
15 inter and intra chromosomal events. Two approaches are considered. In the first, the allotypic
16 differences (i.e. the two chromosomes bear different alleles of the same gene) which can be
17 distinguished by FACS analysis. In the second approach, the two chromosomes are derived from
18 different species, i.e. mouse and human. Hybridoma type fusions between mouse and human lymphoid
19 cells were proposed as the starting point for searching for cells which may have undergone
20 interchromosomal exchange during isotype switching and which would therefore be predicted to appear
21 as mouse-human chimeras.

22 To the best of my knowledge, judged from subsequent studies published by Dr. Herzenberg
23 (including documents M35, M39, M41, Oi *et al.*, "Hybridoma Antibody-Producing Switch Variants:
24 A Variant Lacking the CH1 Domain, pp. 281-287, In: Cell Fusion: Gene Transfer and Transformation,
25 Beers and Basset, eds., Raven Press, New York, 1984 (Exhibit "M"), and Herzenberg *et al.*, J. Cell
26 Biochem. 13th Annual UCLA Symposia, Abstracts, Supplement 8A, 1984, Abstract # 0443 (Exhibit

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1 "N"), it appears that cross-species switch variants have not been detected. Indeed, in view of their later
2 filed patent application (EP 173494A - P3), instead of finding naturally occurring switch variants, in an
3 attempt to make chimeric antibodies Dr. Herzenberg ultimately relied on an approach similar to that
4 published by Dr. Shulman and others, in which a gene was constructed with mouse V region and human
5 C region and transfected into lymphoid cells to obtain expressed antibodies. The grant proposal does
6 not address this method at all. Rather it concentrates on a hypothetical genetic pathway and means for
7 detecting events that would support its existence. It does not instruct one skilled in the art in
8 constructing and expressing chimeric antibodies.

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10 13. In reaching the opinions set forth in this Declaration, I rely upon my education, my
11 research experiences in immunology and biochemistry and my familiarity with the academic and patent
12 literature in my field both today and as existed at the priority date of the Cabilly patent.

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14 14. I declare further that all statements made herein of my own knowledge are true and that
15 all statements made on information and belief are believed to be true; and further that these statements
16 were made with the knowledge that willful false statements and the like so made are punishable by fine
17 or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

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21 Date: 3/14/97

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Jeffrey V. Ravetch, M.D., Ph.D.